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Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal

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Abstract

The aims of this work were to assess the influence of concentration, heat treatment, and pH value on antioxidant activity of ethanolic extracts obtained from Holy basil (*Ocimum sanctum Linn*) and Galangal (*Alpinia galanga*). The antioxidative properties were evaluated. The ethanolic extracts of Holy basil and Galangal showed good heat stability (80 °C, 1 h). At neutral and acidic pH, Holy basil extracts had high antioxidative stability, whereas Galangal extracts showed higher antioxidative stability at neutral than at acidic pH ranges. Antioxidant activity of both extracts at neutral pH was higher than at acidic pH ranges. Holy basil and Galangal extracts exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity, and reducing power in a concentration-dependent manner. Antioxidant activity of both extracts correlated well with reducing power. Furthermore, ethanolic extracts of Holy basil and Galangal acted as radical scavenger and also as lipoxygenase inhibitor.

Keywords: *Ocimum sanctum Linn*; *Alpinia galanga*; Antioxidant activity; Scavenging effect; Chelating effect; Reducing power; Lipoxygenase inhibitory activity

1. Introduction

Oxidative deterioration of fat components in foods is responsible for the rancid odors and flavors which decrease nutritional quality. The addition of antioxidants is required to preserve food quality. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) are widely used as antioxidants in the food industry.

Their safety, however, has been questioned. BHA was shown to be carcinogenic in animal experiments. At high doses, BHT may cause internal and external hemorrhaging, which contributes to death in some strain of mice and guinea pigs. This effect is due to the ability of BHT to reduce vitamin K-depending blood-clotting factor (Ito et al., 1986). Therefore, the importance of replacing synthetic antioxidants by natural ingredients from oil seeds, herbs and spices and other plant materials has increased due to health implications and increased functionality which improves solubility in both, oil and water.

It is well known that natural antioxidants extracted from herbs and spices (rosemary, oregano, thyme, etc.) have high antioxidant activity and are used in many food applications (Hirasa & Takemasa, 1998; Nakatani, 1997). A number of studies deal with the antioxidant activity of extracts from herbs and spices (Cuvelier, Berset, & Richard, 1994; Economou, Oreopoulou, & Thomopoulos, 1991; Kikuzaki & Nakatani, 1993; Lu & Yeap Foo, 2001).

Most of the antioxidative potential in herbs and spices is due to the redox properties of phenolic compounds which allow them to act as reducing agents,

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hydrogen donators and singlet oxygen quenchers (Caragay, 1992; Rice-Evans, Miller, & Paganga, 1997).

Currently, there is an increasing demand for new ethnic foods. New ethnic foods also include the emerging cuisines such as Thai, Vietnamese, Indian and Moroccan, which have strong flavors and aromas. Some of the popular ingredients for developing these foods include tamarind, cardamon, lemon grass, basil, galangal etc. (Cousminer & Hartman, 1996; Uhl, 1996). Galangal (*Alpinia galanga*), a rhizome closely related to the ginger family, is commonly used in stir-fries, curries and soups in Southeast Asia. In fact, it has been reported that galangal, which has gingery notes with slightly sour and peppery notes, is an essential component of Thai curry paste (Uhl, 1996). Several researchers have reported that galangal extract showed antioxidant activity in model system (Barik, Kunda, & Dey, 1987; Cheah & Abu Hasim, 2000; Wang, Chen, Liu, & Guo, 1997). Jitoe et al. (1992) observed that all tropical ginger extracts have antioxidant activities in alcohol/water system.

Basil has been traditionally used in Mediterranean and Southeast Asian foods. Types that are most commonly used in European and American cuisine are local sweet basil, lemon basil, purple ruffle and mintier Egyptian basil. Holy basil (*Ocimum sanctum Linn*), an annual herbaceous plant with slightly hairy, pale green leaves, is widely used as flavouring in Southeast Asian cuisine especially in Thai stirred fries. Holy basil leaves are spicy and have lemony notes (Uhl, 1996). Javanmardi, Stushnoff, Locke, and Vivanco (2003) reported that Iranian basils possess valuable antioxidant properties for culinary and possible medicinal use.

Main objectives of this work were to study the antioxidant properties of the ethanolic extracts from Holy basil and Galangal, including free radical scavenging activity, superoxide anion radical scavenging activity, Fe^{2+} chelating activity, lipoxygenase inhibitory activity, and reducing power. Effects of concentration, heat, and pH on the antioxidant activity of Holy basil and Galangal extracts were also determined.

2. Materials and methods

2.1. Chemicals

Nitroblue tetrazolium (NBT) [No. 2060-67-4], linoleic acid (99%) [No. 60-33-1], 2,2-diphenyl-2-picrylhydrazyl (DPPH) [No. 1898-66-4], 3-(2-Pyridyl)-5-6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine) [No. 2741-96-3], ferrous chloride [No. 10025-77-1], nicotin-amide adenine dinucleotide (NADH) [No. 2101-23-3], ethylenediaminetetraacetic acid (EDTA) [No. 13235-36-4], trichloracetic acid (TCA) [No. 2009-27-2], polyoxyethylenesorbitan monolaurate (Tween 20) [No. 905-64-5] and EC 1.13.11.12 lipoxygenase [No. 2328-53-1] were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents used were of analytical grade and were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany).

2.2. Materials

Fresh Holy basil (*Ocimum sanctum Linn*) leaves and Galangal (*Alpinia galanga*) rhizomes, imported from various locations in Thailand, were purchased from Asian supermarkets in Vienna, Austria. Samples were cleaned, washed with water, cut into small pieces, dried overnight in an air dryer (Memmert-GmbH + Co.KG, type UM 200–800, Germany) at 40 °C, ground to a particle size of 25 mesh by using a grinder (Moulinex, Type MCU 1A, France), and stored at −20 °C in an airtight container until used.

2.3. Extraction procedure

In pre-trials, antioxidative properties such as total phenolic content, reducing power, and antioxidant activity of Holy basil and Galangal were influenced by extraction conditions. Optimum conditions for extraction of antioxidants from Holy basil and Galangal in these trials were the following.

Dried Holy basil powder (4.50 ± 0.05 g dry basis) were extracted by stirring with 50 ml of ethanol and water (3:1, v/v) at 75 °C and 300 rpm for 30 min, whereas, dried Galangal powder (4.5 g dry basis) was extracted with 50 ml of ethanol and water (1:1, v/v) at 50 °C for 1 h. Each extract was then filtered through filter paper (595 1/2 folded filters, ø125 mm, Ref. No. 10311644, Schleicher and Schuell GmbH, Germany); the filtrate was collected and dried by a rotary evaporator (Büchi rotavapor (R), Switzerland) at 40 °C, filled in a plastic bottle and stored at −20 °C until used.

2.4. Properties of Holy basil and Galangal extracts

2.4.1. Effect of concentration

Different concentrations of ethanolic extracts (0.10, 0.25, 0.50, 0.75 and 1.0 mg/ml) were used and antioxidant activity was determined according to the method described by Taga, Miller, and Pratt (1984).

2.4.2. Effect of heat treatment

Dried ethanolic extract (1.00 ± 0.01 g) was placed in a 25 ml beaker and heated in an oven (Memmert-GmbH + Co.KG, type UM 200–800, Germany) at 80 °C for 30 and 60 min, cooled to room temperature, and dissolved in ethanol to produce a final concentration of 1.0 mg/ml. The solution was investigated for antioxidant activity using the β-carotene bleaching method (Taga et al., 1984).

2.4.3. Effect of pH

The influence of pH value on antioxidant activity of dried ethanolic extracts was studied in 0.2 M phosphate buffer with pH values of 3.0, 5.0, and 7.0. The samples were prepared at various pH values as previously mentioned at a final concentration of 1 mg/ml. The antioxidant activity was immediately determined using the β-carotene bleaching method (Taga et al., 1984). To reduce interference on β-carotene caused by pH, a control test was performed using the same buffer without addition of the ethanolic extracts sample. The net absorbance was used to calculate antioxidant activity.

2.4.4. Antioxidative stability at different pH values

Dried ethanolic extracts were dissolved in 0.05 M phosphate buffer at various pH values (3.0, 5.0, and 7.0) and incubated at room temperature for 1 h. Samples were then adjusted with 0.2 M phosphate buffer to a pH of 7.0 and a final concentration of 1 mg/ml before analysis. Antioxidant activity was determined using the β-carotene bleaching method (Taga et al., 1984).

2.5. Antioxidant activity

Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was evaluated by some modification of the method described by Taga et al. (1984). The β-carotene (2 mg) was dissolved in 20 ml of chloroform. A 3 ml aliquot of the solution was put into a 50 ml beaker and 40 mg linoleic acid and 400 mg Tween 20 were added. Chloroform was removed by purging with nitrogen. Oxygenated distilled water (100 ml), which was generated by aerating air bubble into distilled water for 1 h, was added into the β-carotene emulsion and mixed well by using a vortex mixer (Bender and Bobein AG, Model K-550-GE, Switzerland). Aliquots (3 ml) of the oxygenated β-carotene emulsion and 0.12 ml of ethanolic extracts were placed in capped culture tubes and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50 °C. Oxidation of β-carotene emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm in a Hitachi U-1500 spectrophotometer. Absorbance was measured at 0, 10, 20, 30, 40 min. A control was prepared by using 0.12 ml of ethanol instead of the ethanolic extracts. Degradation rate of the extracts was calculated according to first order kinetics using Eq. (1) (Al-Saikhan, Howard, & Miller, 1995)

$$\ln(a/b) \times 1/t = \text{sample degradation rate}, \quad (1)$$

where ln, natural log; a, initial absorbance (470 nm) at time zero; b, absorbance (470 nm) at time 40 min; t, time (min).

The antioxidant activity (AA) was expressed as % inhibition relative to the control using Eq. (2)

$$\text{AA} = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample} \times 100}{\text{Degradation rate of control}}. \quad (2)$$

2.6. DPPH scavenging activity

The effect of the ethanolic extracts on the content of 2,2-diphenyl-2-picrylhydrazyl radical (DPPH·) was estimated according to the modified method of Hatano, Kagawa, Yasuhara, and Okuda (1988). An aliquot (0.5 ml) of the DPPH· solution (50 mg/ml) was diluted in 4.5 ml of methanol, and 0.1 ml of the ethanolic extracts at various concentrations was added. The mixture was shaken vigorously and allowed to stand for 45 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (without extract) in a Hitachi U-1500 spectrophotometer. The decrease in absorbance depends on the antioxidant and radical concentration, the molecular structure of the antioxidant and its kinetic behavior (Brand-Willams, Cuvelier, & Berset, 1995).

From a calibration curve obtained with different amounts of ethanolic extracts, the ED_{50} was calculated. The ED_{50} was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial DPPH· under the experimental conditions given.

2.7. Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of the ethanolic extracts was based on the method described by Liu, Ooi, and Chang (1997). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS–NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT).

In these experiments the superoxide anion was generated in 3 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 μM) solution, 1 ml of NADH (78 μM) solution and different concentrations of the ethanolic extracts (0.10, 0.25, 0.50, 0.75, and 1.0 mg/ml). The reaction started by adding 1 ml of phenazine methosulphate (PMS) solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and its absorbance at 560 nm was recorded against blank samples in a Hitachi U-1500 spectrophotometer. A lower absorbance of the reaction mixture indicated a higher superoxide anion scavenging activity. Superoxide anion scavenging activity (SASA) was calculated using Eq. (3)

$$\text{SASA}[\%] = 1 - \frac{\text{absorbance of sample at 560 nm} \times 100}{\text{absorbance of control at 560 nm}}. \quad (3)$$

2.8. Reducing power

The reducing power of the ethanolic extracts was measured according to the method of Oyaizu (1986). Various concentrations (0.10, 0.25, 0.50, 0.75 and 1.0 mg/ml) of the ethanolic extracts (0.5 ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. TCA (10%: 2.5 ml) was added. The mixture was centrifuged at 650*g* for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride and the absorbance was measured at 700 nm in a Hitachi U-1500 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of the ethanolic extracts was compared with that of L-ascorbic acid (0.2 mg/ml).

2.9. Chelating activity on Fe^{2+}

The chelating activity of the ethanolic extracts on ferrous ions Fe^{2+} was measured according to the method of Decker and Welch (1990). Aliquots of 1 ml of different concentrations (0.10, 0.25, 0.50, 0.75, and 1.0 mg/ml) of the ethanolic extracts were mixed with 3.7 ml of deionized water. The mixture was left for reaction with $FeCl_2$ (2 mM, 0.1 ml) and ferrozine (5 mM, 0.2 ml) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a Hitachi U-1500 spectrophotometer. A lower absorbance indicates a higher chelating power. The Chelating activity on Fe^{2+} of the ethanolic extracts was compared with that of EDTA at a level of 0.01 mM and citric acid at a level of 0.025 M.

Chelating activity was calculated according to Eq. (4)

$$\text{Chelating activity}[\%] = 1 - \frac{\text{absorbance of sample at 562 nm} \times 100}{\text{absorbance of control at 562 nm}}. \quad (4)$$

2.10. Inhibition of lipoxygenase activity

The effect of the ethanolic extracts on lipoxygenase activity was analysed according to the method of Block et al. (1988) with modification. For lipoxygenase analysis, linoleic acid was used as substrate. The substrate was diluted in 0.1 M Tris buffer (pH 8.5) at 22 °C. Different concentrations of ethanolic extracts (10 μl) were added to 1.0 ml of 0.1 M Tris buffer at pH 8 and then 10 μl of lipoxygenase solution in buffer (0.1 μM, final concentration) were added. 25 μl of the substrate (20 μM, final concentration) were added to the mixture, shaken, and allowed to stand at room temperature for 15 min. The decrease in absorbance was then measured at 234 nm against a blank (without enzyme solution) using a Hitachi U-1500 spectrophotometer.

From a calibration curve obtained with different amounts of ethanolic extracts, the IC_{50} was calculated. The IC_{50} was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial lipoxygenase enzyme under the experimental conditions given.

2.11. Statistical analysis

Experimental results were given as mean ± SD of three parallel trials and measurements. P values <0.05 were regarded as significant.

3. Results and discussion

3.1. Effect of concentration of Holy basil and Galangal extracts on antioxidant activity

The antioxidant activity was determined by using a β-carotene/linoleic acid system. In this method a model system of β-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant activity. The free linoleic acid radical formed upon the abstraction of a hydrogen atom from one of its methylene groups attacked the β-carotene molecule, which lost the double bonds and therefore its characteristic orange colour.

The ethanolic extracts from Holy basil and Galangal exhibited effective antioxidant activity at all concentrations studied. The results are shown in Fig. 1. The results indicated that antioxidant activity of Holy basil and Galangal extracts increased with increasing concentration ($P < 0.05$). Only between Holy basil extract at a concentration of 0.75 and 1.0 mg/ml no significant differences in antioxidant activity were observed. The extracts of Holy basil exhibited stronger antioxidant activity than that of Galangal. This was postulated to be due to different antioxidative compounds in both ex-

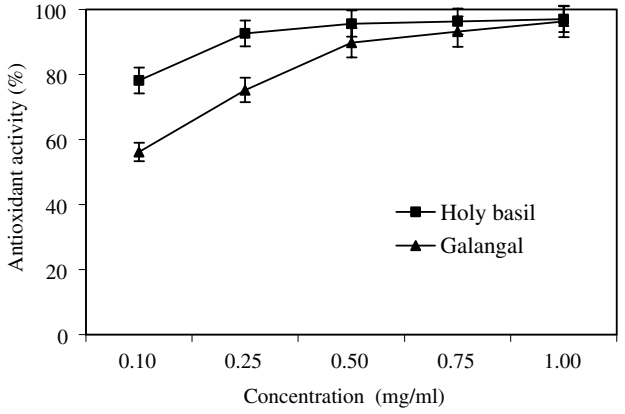

Fig. 1. Antioxidant activity of Holy basil and Galangal extracts at different concentrations.

tracts. This result was in agreement with Yen and Lee (1997) who found that antioxidant activity of the extract from *Aspergillus candidus* broth filtrate increased with increasing concentration, but it reached maximum after 200 ppm. Similarly, Duh, Yen, Du, and Yen (1997) observed that antioxidant activity of mung bean hull extract increased with increasing concentration up to 100 ppm and no significant differences in antioxidant activity were observed with concentration ranging from 100 to 500 ppm ($P < 0.05$).

3.2. Effect of heat treatment on antioxidant activity of Holy basil and Galangal extracts

The ethanolic extracts from Holy basil and Galangal were heated at 80 °C for 0, 30 and 60 min, and the residual antioxidant activity was determined by the β-carotene bleaching method. Fig. 2 shows the antioxidant activity of Holy basil extracts as a function of heating time. Heating at 80 °C for 0, 30 and 60 min did not significantly reduce antioxidant potency ($P > 0.05$). Results of this study demonstrated that antioxidant activity of Holy basil and Galangal extracts was heat stable, even after heating at 80 °C for 60 min. Tsuda, Osawa, Nakayama, Kawakishi, and Ohshima (1993) reported that antioxidant activity of pure compound from pea bean extract was completely heat stable, even after heating for 1 h at 100 °C. Lee, Kim, and Ashmore (1986) reported that the extract of ginger rhizome was also heat stable. Two thirds (relative antioxidant potency = 67.8) of the original antioxidant activity still remained after 2 h at 100 °C. Similarly, Rehman, Salariya, and Habib (2003) found that Ginger extract showed good thermal stability and exhibited 85.2% inhibition of peroxidation of linoleic acid when heated at 185 °C for 120 min. Furthermore, antioxidant activities of cocoa by-product extracts were stable up to 50 °C, the antioxidant activities dropping significantly at 70–90 °C (Abdul Hamid, Nik Muhammad, & Thed, 1999).

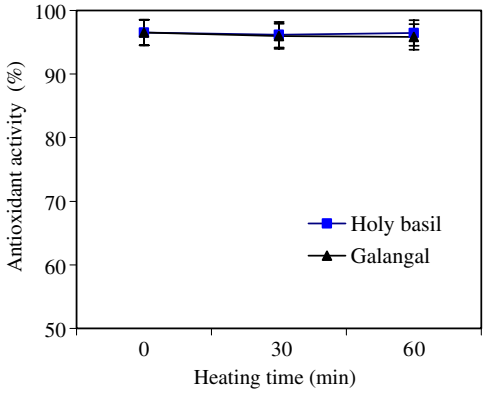

Fig. 2. Antioxidant activity of Holy basil and Galangal extracts as a function of heating time at 80 °C and a concentration of 1 mg/ml.

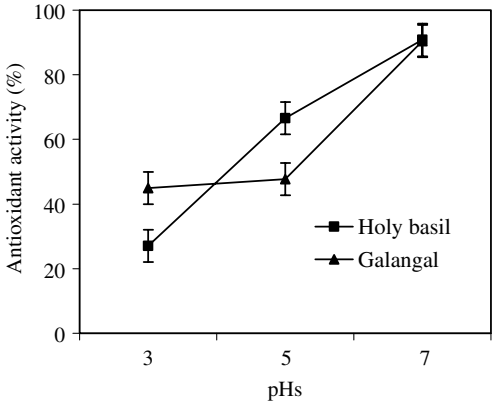

Fig. 3. The effect of pH value on antioxidant activity of Holy basil and Galangal extracts at a concentration of 1 mg/ml.

3.3. Effect of pH value on antioxidant activities of Holy basil and Galangal extracts

The Holy basil and Galangal extracts showed strong antioxidant activity at neutral pH and weak activity at acidic pH (Fig. 3). At pH 3.0, the ethanolic extracts from Holy basil and Galangal exhibited lowest antioxidant activity while the highest antioxidant activity was found at pH 7.0. Yen and Duh (1993) and Yen and Lee (1997) reported that the extracts from peanut hull and *Aspergillus candidus* broth filtrate exhibited strong antioxidant activity at neutral pH and acidic pH range but rendered no activity at alkaline pH ranges. Tsuda, Ohshima, Kawakishi, and Osawa (1994) found that anthocyanin from red and black beans showed different antioxidant activity, depending on pH. The difference in antioxidant activity of Holy basil and Galangal extracts at various pH values is possibly due to the different conformation and charges of antioxidant compounds under different pH values.

3.4. Antioxidative stability of Holy basil and Galangal extracts at different pH values

Holy basil and Galangal extracts had high antioxidative stability at pH 7.0 (Fig. 4). The antioxidative stability of ethanolic extracts from Holy basil was slightly decreased in acidic pH ranges, whereas that of Galangal was greatly decreasing in acidic pH ranges. The results indicated that Holy basil and Galangal extracts had high antioxidative stability at neutral pH ranges. Acidic condition was able to change the antioxidant property of extracts.

3.5. DPPH scavenging activity

It is well known that free radicals cause autoxidation of unsaturated lipids in food (Kaur & Perkins, 1991). In addition, antioxidants are known to interrupt the

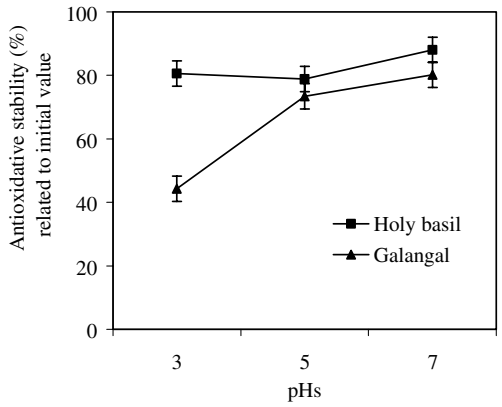

Fig. 4. Antioxidative stability of Holy basil and Galangal extracts at a concentration 1 mg/ml for 1 h incubation at different pH values.

free-radical chain of oxidation and to donate hydrogen from phenolic hydroxy groups, thereby, forming stable free radicals, which do not initiate or propagate further oxidation of lipids (Sherwin, 1978). Recent reports have described antioxidants and compounds with radical scavenging activity present in onion, garlic, sage, and thyme extracts (Miura, Kikuzaki, & Nakatani, 2002; Nuutila, Pimia, Aarni, & Caldentey, 2003). Therefore, radical scavenging of Holy basil and Galangal extracts was determined. The 2,2-diphenyl-2-picrylhydrazyl radical has been widely used to evaluate the free radical scavenging capacity of antioxidants (Brand-Willams et al., 1995; Espin, Soler-Rivas, & Wichers, 2000; Yu, 2001). The determination of scavenging stable DPPH· was a very fast method to evaluate the antioxidant activity of the extracts. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH· at 515 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH· was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH· molecule. In the radical form this molecule had an absorbance at 515 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthäus, 2002).

ED_{50} is the concentration of ethanolic extracts from Holy basil and Galangal to quench 50% DPPH· under the chosen experimental conditions. As shown in Table 1, the ethanolic extracts from Holy basil showed better scavenging capacity than the ethanolic extracts from Galangal. The ED_{50} of Holy basil and Galangal extracts to quench DPPH· were 0.30 and 0.42 mg extract/ml, respectively. Matthäus (2002) reported that extracts of sunflower residues between 0.15 and 1.16 mg were necessary to reduce the DPPH· by 50%. Yu et al. (2002) found that the ED_{50} values of wheat extract against DPPH· were 0.60 mg/ml for Akron, 7.1 mg/ml for Trego and 0.95 mg/ml for Platte. Jiménez-Escrig, Jiménez-Jiménez, Sánchez-Moreno, and Saura-Calixto (2000) reported that the ED_{50} of the carotenoids lycopene, β-carotene, β-cryptoxanthin, lutein, α-carotene and zeaxanthin reduce the initial DPPH· concentration to 50%, ranging from 0.16 to 3.29 mol carotene/mol DPPH·.

Table 1
ED_{50} of Holy basil and Galangal extracts against DPPH·[a] and lipoxygenase inhibitory activity (IC_{50})[b] of Holy basil and Galangal extracts

Extracts	ED_{50} (mg extract/ml)	IC_{50} (μg extract/ml)
Holy basil	0.30	20.6
Galangal	0.42	27.5

[a] ED_{50} is the concentration of Holy basil and Galangal extracts to quench 50% DPPH· under the chosen experimental conditions.
[b] IC_{50} is the concentration of Holy basil and Galangal extracts added that inhibits 50% lipoxygenase activity under the chosen experimental conditions.

The data obtained, reveal that both ethanolic extracts from Holy basil and Galangal are free-radical inhibitors, thus primary antioxidants that react with free radicals. This property may be the main factor to cause inhibition of peroxidation of linoleic acid. Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reactions and reducing power (Shimada, Fujikawa, Yahara, & Nakamura, 1992; Tanaka, Kuie, Nagashima, & Taguchi, 1988).

3.6. Superoxide anion scavenging activity

Superoxide anions ($\cdot O_2$), the one-electron reduced form of molecular oxygen, is a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage (Halliwell & Gutteridge, 1984). Dahl and Richardson (1978) noted that superoxide decomposes to form stronger oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Meyer and Isaksen (1995) reported that superoxide anion indirectly initiates lipid oxidation as a result of superoxide and hydrogen peroxide serving as precursors of singlet oxygen and hydroxyl radicals. In the PMS–NADH–NBT system, superoxide anion derived from dissolved oxygen by PMS–NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture.

Superoxide anion scavenging activity of Holy basil and Galangal extracts at different amounts is illustrated in Fig. 5. Results showed that superoxide anion scavenging activity of both extracts was increased with an increasing amount of the extracts. Superoxide anion scavenging activity of Holy basil extracts was higher than that of Galangal extracts at the same concentra-

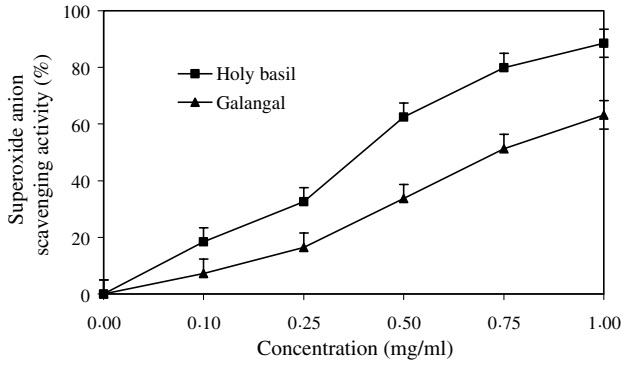

Fig. 5. Superoxide anion scavenging activity of Holy basil and Galangal extracts at different concentrations.

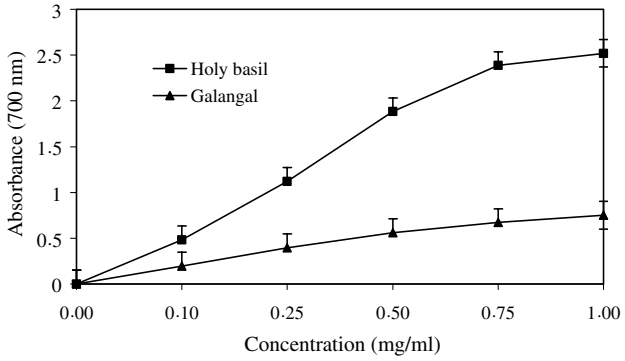

Fig. 6. Reducing power of ethanolic extracts from Holy basil and Galangal at different concentrations.

tion. Superoxide anion scavenging activity of Holy basil and Galangal extracts at an amount of 1.0 mg/ml was 88.51 ± 1.15% and 63.22 ± 1.15%, respectively. This superoxide radical scavenging potential of extracts is supposed to one of the major mechanisms contributing to their antioxidant capacity.

Recent studies have shown that phenolic compounds, particularly flavonoids and catechins, are important antioxidants and superoxide scavengers. Their scavenging efficiency depends on the concentration of phenol and the numbers and locations of the hydroxyl groups (Benavente-Garia, Castillo, Marin, Ortuno, & Del-Rio, 1997). Gülçin, Oktay, Küfrevioğlu, and Aslan (2002) found that the superoxide anion radical scavenging activity of *Cetraria islandica* L. depended on concentration and was increasing with increased amount of sample. Nagai, Inoue, Inoue, and Suzuki (2003) noted that the scavenging activity against the superoxide anion radical of aqueous propolis extract was high, and the extracts, at 50 and 100 mg/ml, completely inhibited the production of superoxide.

3.7. Reducing power

During the reducing power assay, the presence of reductants (antioxidants) in the tested samples would result in reducing Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung, Chang, Chao, Lin, & Chou, 2002). Fig. 6 shows the reducing power of ethanolic extracts from Holy basil and Galangal. The reducing power (as indicated by the absorbance at 700 nm) of Holy basil and Galangal extracts rose with an increase in concentration and was 2.52 and 0.75 at 1.0 mg/ml, respectively. At the same concentration levels, Holy basil extracts showed higher reducing power than Galangal extracts. In addition, the reducing power of Holy basil extracts with concentrations ranging from 0.10 to 1.0 mg/ml was higher than that of L-ascorbic acid at a concentration level of 0.2 mg/ml (reducing power

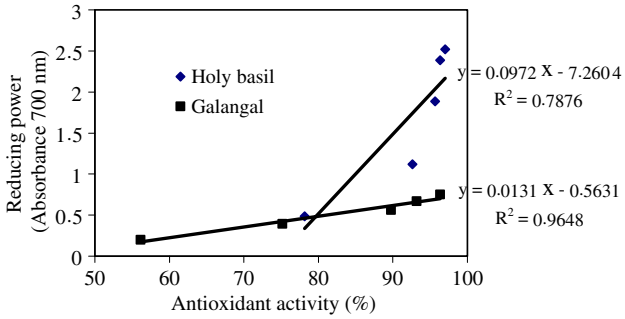

Fig. 7. Relationship between antioxidant activity and reducing power of Holy basil and Galangal extracts.

of 0.72). In contrast, reducing power of Galangal extracts at a concentration level of 1.0 mg/ml was similar to that of as compared to L-ascorbic acid at a concentration level of 0.2 mg/ml.

The antioxidant activity has been reported by some investigators to be concomitant with the development of reducing power (Duh, 1998; Yen & Duh, 1993). Antioxidant activity and reducing power of Holy basil and Galangal extracts increased with an increasing amount of the extracts. Correlation coefficients between antioxidant activity and reducing power for Holy basil and Galangal extracts were high (r^2 = 0.9648 and 0.7876, respectively) (Fig. 7), indicating that antioxidant properties in Holy basil and Galangal extracts were concomitant with the development of reducing power. This result was in agreement with that of Yen and Duh (1993), who reported that the reducing power of peanut hull extract increased with increase in concentration and correlated (r^2 = 0.9793) well with the extent of antioxidant activity. Similarly, Duh (1998) and Duh et al. (1997) found that the antioxidant properties of mung bean hull and burdock extracts were shown to be concomitant with the development of reducing power. Gordon (1990) reported that the antioxidant action of reductones is based on breaking of the radical chain by donation of a hydrogen atom.

3.8. Chelating activity on Fe^{2+}

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxidase to reactive free radicals via the Fenton type reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH°$). Fe^{3+} ion also produces radicals from peroxides, although the rate is tenfold less than that of Fe^{2+} ion (Miller, 1996). Fe^{2+} ion is the most powerful pro-oxidant among various species of metal ions (Halliwell & Gutteridge, 1984). Ferrozine, a chelating reagent, was used to indicate the presence of chelator in the reaction system. Ferrozine formes a complex with free Fe^{2+} but not with Fe^{2+} bound by extracts. In the presence of chelating agents, the complex formation of ferrous and ferrozine is disrupted, resulting in a decrease in red color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the coexisting chelator (Yamaguchi, Ariga, Yoshimira, & Nakazawa, 2000).

In this study, measurement of chelating activity on Fe^{2+} of Holy basil and Galangal extracts was done using ethanolic extracts ranging from 0.10 to 1.0 mg/ml. The Holy basil and Galangal extracts showed chelating activity on Fe^{2+} in a concentration-dependent manner (Fig. 8). At the same amount Holy basil extracts exhibited higher chelating activity on Fe^{2+} than Galangal extracts. However, chelating activity of Holy basil extracts with concentrations of 0.75 and 1.0 mg/ml was higher than that of EDTA at 0.01 mM and citric at acid 0.025 M (33.31% and 32.05%, respectively), whereas Galangal extracts within all studied concentrations exhibited lower chelating activity than EDTA at 0.01 mM and citric acid at 0.025 M.

Mau et al. (2003) reported that the chelating effect of an essential oil from *Curcuma zedoaria* on ferrous ion was 8.7% at 0.01 mg/ml and increased to 23.2% at 20 mg/ml, which is slightly lower than that of citric acid (25.2%) and much lower than that of EDTA at 1.0 mg/ml (99.5%). Chen and Ahn (1998) found that natural phenolics including quercetin, rutin, catechin and caffeic acid acted as Fe^{2+}-chelators. The data obtained revealed that Holy basil and Galangal extracts functioned as Fe^{2+}-chelators. Holy basil and Galangal extracts either chelated metal ions or suppressed reactivity by occupying all coordination sites of the metal ion (Mahoney & Graf, 1986). Therefore, it can be used as an effective agent in retarding Fe^{2+}-catalyzed lipid oxidation.

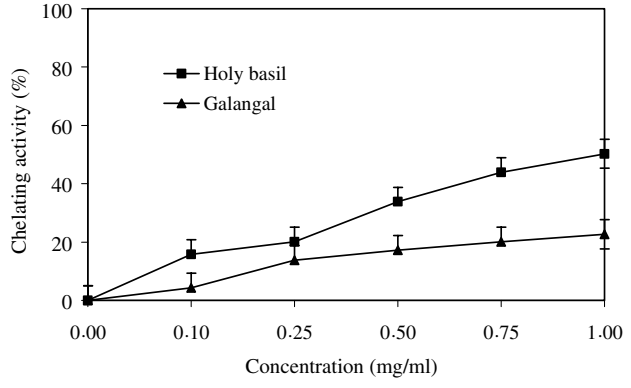

Fig. 8. Chelating activity on Fe^{2+} of Holy basil and Galangal extracts at different concentrations.

3.9. Inhibition of lipoxygenase activity

Lipoxygenase catalyzes oxygenation of polyunsaturated fatty acid containing a *cis*,*cis*-1-4-pentadiene system to hydroperoxides (Nawar, 1985). The lipoxygenase pathway of arachidonic metabolism produces reactive oxygen species, and these reactive forms of oxygen and other arachidonic acid metabolites may play a role in inflammation and tumor promotion. Inhibitors of arachidonic acid metabolism also inhibited tumor promotion in animal models (Sreejayan & Rao, 1996). Antioxidants are generally known to inhibit plant lipoxygenases (Ammon, Annazodo, Safayhi, Dhawan, & Scrimal, 1992). Therefore an attempt was made to study the lipoxygenase inhibitory activities of Holy basil and Galangal extracts.

The IC_{50} of Holy basil and Galangal extracts to quench lipoxygenase were 20.63 and 27.52 μg/ml, respectively (Table 1). IC_{50} is the concentration of extracts which inhibited 50% lipoxygenase activity under the chosen experimental conditions. Thus, Holy basil extracts showed higher lipoxygenase inhibitory activity than Galangal extracts. Chen, Shi, and Ho (1992) found that the IC_{50} of rosemary extract to quench soybean 15-lipoxygenase ranged from 1.3 to 2.6 μg. Five isoflavone and isoflavone glycosides isolated from kudzu root acted as lipoxygenase inhibitory activity, i.e., puerarin, daidzin, daidzein, biochanin A and genistein (Jun et al., 2003). Lipoxygenase, an enzyme that specially introduces oxygen into free fatty acids, contains iron within its molecular structure (Shimoni, Armon, & Neeman, 1994). From the before mentioned results it could be seen that Holy basil and Galangal extracts acted as Fe^{2+}-chelators. Probably, Holy basil and Galangal extracts inhibited lipoxygenase activity via interaction with a ferric ion at the active site, leading to enzyme inactivation.

4. Conclusion

This study showed that ethanolic extracts produced from Holy basil and Galangal exhibited good heat stability (80 °C, 1 h). Holy basil extracts had high antioxidative stability at neutral and acidic pH. Galangal

extracts had high antioxidative stability at neutral pH, but the antioxidative stability in acidic pH ranges was lower. Antioxidant activity of Holy basil and Galangal extracts was higher at pH 7 than that at acidic pH ranges. Antioxidant activity of Holy basil extracts increased with increasing concentrations, ranging from 0.10 to 0.75 mg/ml and then reached a plateau at concentrations ranging from 0.75 to 1.0 mg/ml. Antioxidant activity of Galangal extracts increased with increasing concentration, ranging from 0.10 to 1.0 mg/ml. In addition, antioxidant activity of Holy basil and Galangal extracts was concomitant with the development of reducing power. The ethanolic extracts of Holy basil and Galangal exhibited strong superoxide anion scavenging activity, Fe^{2+} chelating activity, and reducing power in a concentration-dependent manner, and additionally acted as radical scavengers and lipoxygenase inhibitors.

From the results it can be concluded, that Holy basil showed higher antioxidant properties than Galangal extracts, presumably due to differences in structure of the antioxidant components. However, both ethanolic extracts could be used as natural food antioxidants and a possible substitution of artificial antioxidants should be considered. Besides their natural properties, they have the advantages of being easy to obtain, cheap, and effective. Therefore, it would be interesting to do further studies of using Holy basil and Galangal extracts as food additives in order to increase the shelf life of foods by preventing lipid peroxidation.

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